

PATENT  
10/039,956  
Docket 091/009c

CLAIM AMENDMENTS

1. *(Original)* A composition comprising proliferating primate pluripotent stem (pPS) cells, which is essentially free of feeder cells.

2 to 10. *CANCELLED*

11. *(Original)* A method for producing differentiated cells from a donor culture of undifferentiated primate pluripotent stem (pPS) cells, comprising:
  - a) preparing a suspension of cells from the undifferentiated donor culture;
  - b) replating and culturing the suspended cells on a solid surface so that they differentiate without forming embryoid bodies; and
  - c) harvesting differentiated cells from the solid surface.
12. *(Original)* A method for producing differentiated cells from a donor culture of primate pluripotent stem (pPS) cells, comprising:
  - a) providing a culture of primate pluripotent stem (pPS) cells that is essentially free of feeder cells;
  - b) changing the medium in which the cells are cultured; and
  - c) harvesting differentiated cells after culturing for a time in the changed medium.
13. *(Previously presented)* The method of claim 11, wherein the donor culture of pPS cells is a culture essentially free of feeder cells, according to claim 1.
14. *(Original)* The method of claim 11, having at least one of the following features:
  - i) the solid surface bears a poly-cation (such as poly-lysine or poly-ornithine);
  - ii) differentiation is promoted by withdrawing serum, serum replacement, or a factor that inhibits differentiation from medium in which the cells are cultured after replating; or
  - iii) differentiation is promoted by adding a factor (such as Brain Derived Neurotrophic Factor, BDNF; or Neurotrophin-3, NT-3) that promotes differentiation in medium in which the cells are cultured after replating.
15. *(Previously presented)* A differentiated cell population produced by the method of claim 35.

PATENT  
10/039,956  
Docket 091/009c

16. *(Currently Amended)* A method of screening a compound for cellular toxicity or modulation substance, comprising contacting a differentiated cell according to claim 15 with the compound, with the substance, and determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with cellular toxicity or modulation substance.
17. *(Original)* A method for producing a polynucleotide comprising a nucleotide sequence contained in an mRNA that is expressed at a different level in committed or differentiated cells compared with undifferentiated primate pluripotent stem (pPS) cells, the method comprising:
  - a) determining the level of expression of a plurality of mRNAs in committed or differentiated cells, in comparison to the level of expression of the same mRNAs in undifferentiated pPS cells;
  - b) identifying an mRNA expressed at a different level in the committed or differentiated cells, relative to the undifferentiated pPS cells; and
  - c) preparing a polynucleotide comprising a nucleotide sequence of at least 30 consecutive nucleotides contained in the identified mRNA.

18 to 29. CANCELLED

30. *(Previously Presented)* A method for producing a protein containing a sequence of a polypeptide expressed in undifferentiated or differentiated pPS cells, comprising determining amino acid sequence from a protein encoding region of an mRNA or cDNA from an expression library, and manufacturing a protein containing the determined sequence;  
wherein the expression library was obtained by providing a culture of undifferentiated pPS cells essentially free of feeder cells, optionally permitting the pPS cells to differentiate, and isolating mRNA from the undifferentiated or differentiated cells.
31. *(Previously Presented)* A method for producing an antibody specific for a polypeptide expressed in undifferentiated or differentiated pPS cells, comprising determining amino acid sequence from a protein encoding region of an mRNA or cDNA from an expression library, and immunizing an animal or contacting an immunocompetent cell or particle with a protein containing the determined sequence;  
wherein the expression library was obtained by providing a culture of undifferentiated pPS cells essentially free of feeder cells, optionally permitting the pPS cells to differentiate, and isolating mRNA from the undifferentiated or differentiated cells.
32. *(Original)* The composition of claim 1, wherein the pPS cells are human embryonic stem (hES) cells.

PATENT  
10/039,956  
Docket 091/009c

33 to 34. **CANCELLED**

35. *(Currently Amended)* A method for producing a population of differentiated cells, comprising:  
a) obtaining a line of embryonic stem cells primate pluripotent stem (pPS) cells that have been established in a culture environment that is essentially free of feeder cells; and  
b) optionally causing or permitting cells in the culture to differentiate into the population of differentiated cells.
36. **CANCELLED**
37. *(Currently Amended)* ~~A method of screening a substance for its effect on cultured cells. The method of claim 16,~~ comprising:  
a) obtaining a culture of undifferentiated pPS cells proliferating in a growth environment that is essentially free of feeder cells;  
b) optionally causing or permitting the pPS cells to differentiate; then  
c) combining the cells with the substance; and  
d) determining any effect of the substance on the cells.
38. *(Previously Presented)* The method of claim 37, wherein the undifferentiated pPS cells are cultured on extracellular matrix components (such as Matrigel®, laminin, or collagen) in the absence of feeder cells.
39. *(Previously Presented)* The method of claim 37, wherein the cells are undifferentiated when contacted with the substance.
40. *(Previously Presented)* The method of claim 37, wherein the cells have been caused or permitted to differentiate before being contacted with the substance.
41. *(Previously Presented)* The method of claim 40, wherein the cells have been caused to differentiate by a process comprising replating them onto a surface that promotes differentiation.
42. *(Previously Presented)* The method of claim 40, wherein the cells have been caused to differentiate by adding component(s) to the medium that promote differentiation towards a particular cell lineage.
43. *(Previously Presented)* The method of claim 40, comprising causing the cells to differentiate into cells having characteristics of neuronal cells, glial cells, or neural precursors.

PATENT  
10/039,956  
Docket 091/009c

44. *(Previously Presented)* The method of claim 40, comprising causing the cells to differentiate into cells having characteristics of hepatocytes.
45. *(Previously Presented)* The method of claim 37, wherein the pPS cells are human embryonic stem (hES) cells.
46. *(Previously Presented)* The method of claim 37, comprising determining the effect of the substance on growth of the cells.
47. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects differentiation of the cells.
48. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects expression of a marker or receptor by the cells.
49. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects release of a marker or enzyme from the cells.
50. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects DNA synthesis or repair in the cells.
51. *(Previously Presented)* The method of claim 37, comprising analyzing the cells by metaphase spread.
52. *(Previously Presented)* The method of claim 37, comprising determining whether the compound is toxic to the cells.